

Remarks

Claims 1-38 are pending. Claims 11, 12, 18-22 and 29-36 are withdrawn. Claims 1-10, 13-17, 23-28, 37 and 38 are rejected. Claim 1 is currently amended. Claim 28 is cancelled. Support for the amendments to Claim 1 can be found at, for example, paragraphs [0006], [0032], [0035] and the abstract of the originally filed application. The Applicants also wish to thank the Examiner for removing the previous rejections.

Claims 1-5, 8, 10, 14-17, 23-27 and 37-38 are rejected as obvious under 35 USC §103(a) over the combination of US '552 and Zierdt.

Amended Claims 1-5, 8, 10, 14-17, 23-27 and 37-38 are not obvious under 35 USC §103(a) over the combination of US '552 and Zierdt. Independent Claim 1 has been amended to include the recitation that the steps of the method are "performed in an enclosed and sterile device." Claims 2-5, 8, 10, 14-17, 23-27 and 37-38 are dependent on independent Claim 1.

Neither US '552 nor Zierdt teach steps "performed in an enclosed and sterile device" and do not disclose a device allowing one of ordinary skill in the art to do this. In other words, the technical feature that distinguishes the subject matter of the amended claims from US '552 and Zierdt is that the method is performed "in an enclosed and sterile device." Thus, amended Claims 1-5, 8, 10, 14-17, 23-27 and 37-38 are not obvious over the combination of US '552 and Zierdt because this combination of references fails to teach all the elements of the amended claims.

Indeed, the Applicants' methods permit contaminating microbes present in blood to be concentrated and labeled without any risk of producing erroneous results via the inadvertent introduction of additional microbes not originally present in a blood sample being analyzed. This means that in the claims the labeled contaminating microbes are those present in the original blood sample, and not microbes coming from other sources into a blood sample. Consequently, the claimed methods allow the detection of contaminating microbes in a blood sample without any external contamination.

For example, US '552 indicates that it is desirable to provide means for removing substantially all of the red blood cells from a sample of whole blood. *See* US '552 at column 7, lines 24-26. In US '552, an agglutinating agent is used to form red blood cell clusters that may be removed by any suitable means such as filtration. *See* US '552 at column 11, lines 40-41. In US '552 the preferred filtration means is a porous absorbent pad which can be impregnated with

an agglutinating agent. *See* US '552 at column 11, lines 50-53 and the mesh or pore size of the pad are preferably from about 20 to 500 microns (column 11, lines 62-64). In US '552, a second filter may be used to trap any extra red blood cells that may escape from the absorbent pad. *See* US '552 at column 12, lines 2-3. In US '552, the secondary filter should have a very small pore size, allowing plasma to pass through this filter while retaining any residual red blood cells. Lastly, in US '552, the pore size of this second filter is ideally between 1 and 5 μm . *See* US '552 at column 12, lines 8-11.

Importantly, US '552 does not teach anything about a method "performed in an enclosed and sterile device" for recovering microbes in a sample and detecting labeled microbes in the sample without any extraneous microbe introduction risk.

Zierdt discloses a method wherein a lysing solution is used to disrupt phagocytes such that intracellular bacteria are released from the lysed phagocytes. A filtration step permits the retention of the released formerly intracellular bacteria which may then be detected. In Zierdt, the pores on this filter membrane are 0.45 μm which allow retention of these intracellular bacteria and the passage of cellular debris. The lysis-filtration culture technique of Zierdt allows a greater sensitivity to detect bacteria.

Importantly, Zierdt does not teach anything about a method "performed in an enclosed and sterile device" for recovering microbes in a sample and detecting labeled microbes in the sample without any extraneous microbe introduction risk.

Furthermore, performing a labeling step, such as step d), simultaneously with a separating step, such as an aggregation step b) or lysing step c), has not been suggested by the cited art. Certainly, the cited art does not teach simultaneously performing these steps "in an enclosed and sterile device." Additionally, the problem of subsequent microbial contamination of samples is not mentioned in the cited references and no solution to this problem is provided by this art. This means that one of ordinary skill in the art would not be motivated to combine the teachings of US '552 and Zierdt.

The above makes it clear that amended claims Claims 1-5, 8, 10, 14-17, 23-27 and 37-38 should be considered patentable over the combination of US '552 and Zierdt on the basis that none of the cited art discloses or suggests a method for detecting contaminating microbes possibly present in a blood product comprising blood cells comprising a) subjecting a sample of the blood product to an aggregation treatment of the blood cells, b) substantially eliminating

aggregates formed in step (a) by passage of the sample over a first filter allowing passage of contaminating microbes, but not cell aggregates, c) selectively lysing residual cells of the filtrate obtained in step (b), d) adding a marker agent to label the contaminating microbes either during step (a) or step (c), e) recovering the contaminating microbes by passage of the lysate from step (c) over a second filter with a pore size of about 0.3 μm to less than 1 μm which retains contaminating microbes and allows passage of cellular debris, and f) analyzing material on the second filter to detect labeled contaminating microbes possibly retained by the second filter said method being performed in an enclosed and sterile device.

Claims 6 and 7 are rejected as obvious under 35 USC §103(a) over the combination of US '552, Zierdt, and US '215. Claims 9 and 13 are also rejected as obvious under 35 USC §103(a) over the combination of US '552, Zierdt, and US '925.

Claims 6 and 7 are not obvious over the combination of US '552, Zierdt, and US '215. Claims 9 and 13 are not obvious over the combination of US '552, Zierdt, and US '925. This is because US '215 and US '925 do nothing to correct the deficiencies of the core combination of US '522 and Zierdt with respect to these claims. These deficiencies are discussed in detail above.

The Applicants respectfully request withdrawal of the rejections of Claims 1-10, 13-17, 23-27, 37 and 38 as obvious.

In light of the foregoing, the Applicants respectfully submit that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,



T. Daniel Christenbury
Reg. No. 31,750
Attorney for Applicants

TDC/vbm
(215) 656-3381